

Relieving phantom limb pain with multimodal sensory-motor training

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Title page

Title: Relieving Phantom Limb Pain with Multimodal Sensory-Motor Training

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1 **Abstract:**

2 *Background*

3 The causes for the disabling condition of phantom limb pain (PLP), affecting 85% of amputees, are so far
4 unknown, with few effective treatments available. Sensory feedback based strategies to normalize the motor
5 commands to control the phantom limb offer important targets for new effective treatments as the correlation
6 between phantom limb motor control and sensory feedback from the motor intention has been identified as a
7 possible mechanism for PLP development.

8 *Methods*

9 Ten upper-limb amputees, suffering from chronic PLP, underwent 16 days of intensive training on phantom-
10 limb movement control. Visual and tactile feedback, driven by muscular activity at the stump, was provided
11 with the aim of reducing PLP intensity.

12 *Results*

13 A 32.1% reduction of PLP intensity was obtained at the follow-up (6 weeks after the end of the training, with
14 an initial 21.6% reduction immediately at the end of the training) reaching clinical effectiveness for chronic
15 pain reduction.

16 *Conclusion*

17 Multimodal sensory-motor training on phantom-limb movements with visual and tactile feedback is a new
18 method for PLP reduction.

19 *Significance*

20 The study results revealed a substantial reduction in phantom limb pain intensity, obtained with a new training
21 protocol focused on improving phantom limb motor output using visual and tactile feedback from the stump
22 muscular activity executed to move the phantom limb.

23

1 **Introduction**

2 Phantom Limb Pain (PLP) is pain felt in the missing limb in amputees and represents an extremely
3 challenging pain condition to treat. Regardless of the reason for amputation, the prevalence of PLP is reported
4 to be as high as 85% (1, 2). Previous studies have suggested that the primary sensorimotor cortex (SM1)
5 undergoes a reorganisation involving both structural (3-5) and functional (6-11) changes contralateral to the
6 amputation. These changes are believed to result from the loss of afferent input, allowing for invasion of
7 neighbouring cortical regions into the former limb representation area in the SM1.

8 Another aspect that has been extensively investigated is sensory-motor incongruence (12-14),
9 implying that pain, in the absence of ongoing tissue damage, might be caused by incongruence between motor
10 intention and proprioceptive feedback (14, 15). According to Ramachandran (12), when a limb is intact, motor
11 commands to move a limb are usually damped by sensory error feedback, such as vision and proprioception
12 (16, 17). With a phantom limb, this damping effect is not present and the motor output may become amplified
13 and experienced as painful (18, 19). Training with visual feedback on the phantom movements driven by the
14 motor activity of the stump may possibly dampen the painful and uncontrolled motor output, decreasing
15 phantom-limb pain (20, 21). Therefore, mirror therapy, graded motor imagery, tactile training or sensory
16 discrimination may correct cortical body maps by removing the incongruence between motor commands and
17 sensory feedback (20).

18 The motor capacities of the phantom limb are of interest for PLP treatment because there is some
19 evidence of a relation between the ability to control movements of the phantom limb and the severity of PLP
20 (22-25). This is supported by the clinical observation that many amputees feel that their phantom limb is fixed
21 in one position and report cramping sensations as one of the main characteristics of their phantom pain (22,
22 24). Moreover, activation of remnant muscles in the stump is impaired in patients with pain relative to
23 amputees who are pain free (22), where PLP participants show slower cyclic phantom movements with a
24 higher degree of muscular modulation compared to the pain-free amputees (22). The interaction between
25 central motor commands and sensory feedback in the perception of phantom movement was further

1 demonstrated by Reilly and colleagues (26). In an amputee with a frozen phantom limb, they observed that the
2 stump-muscle activity did not vary when attempting to perform different movements of the phantom limb.
3 This behaviour could also be induced in amputees able to differentiate muscle activity of their stump
4 following ischemic nerve block. Therefore, there is an established link between PLP and the motor control of
5 phantom movements showed by the positive correlation between the amount of electromyography (EMG)
6 activity acquired in remnant stump muscles during phantom limb movements and the intensity of the phantom
7 pain (22). This connection supports the idea of training phantom limb motor control with direct feedback of
8 the different muscular effort of the residual limb muscles.

9 However, few effective treatments are available for PLP (10). Some are invasive, such as local
10 anaesthesia, sympathectomy and rhizotomy, and therefore not always accepted by patients. Pharmacological
11 interventions, such as anticonvulsants, neuroleptics and muscle relaxants, may lead to side effects that
12 negatively impact on quality of life (27). Current non-pharmacological/non-invasive approaches, such as
13 mirror therapy (28, 29), provide visual feedback in order to counteract sensorimotor incongruence but show
14 hindered efficacy in case of limited integration between sensory feedback and the kinaesthetic sensation of the
15 phantom (e.g. shrunk in case of telescoping) (30). Virtual visual feedback during phantom movement to
16 alleviate phantom pain has been used in previous studies (24, 31, 32) and the results are encouraging.
17 However, controlled studies with larger samples are needed to determine which patients are most likely to
18 benefit from such virtual feedback therapy (33, 34). Somatosensory feedback provided by electro-tactile
19 stimulation has been proposed to reduce PLP in a very homogeneous patient group of transradial myoelectric
20 prosthesis users (35). Sensory discrimination training programmes, based on electrical or mechanical tactile
21 stimuli have demonstrated a significant reduction in PLP (upper and lower limb amputees) (36, 37).
22 Moreover, in a recent clinical trial (38), augmented reality was used to decrease the PLP intensity, using
23 visual data as the only source of sensory feedback.

24 There are no reports exploring multimodal sensory feedback substitution associated with phantom
25 motor execution for PLP relief. In the current study, we devised and tested a new phantom-limb movement

1 training protocol focused on providing visual and tactile feedback substitution in upper-limb amputees
2 suffering from chronic PLP with the main aim of reducing PLP intensity. Volitional control of the phantom
3 limb was used as the principal component of the proposed treatment, sustained by visual and tactile feedback
4 of the EMG activity generated by the remnant muscles of the residual limb during the execution of the
5 phantom movements. The participants were asked to train the execution of specific wrist, hand and finger
6 movements with their phantom and to learn to control the muscular effort to accomplish such movements.
7 Participants were able to modulate the motor output using visual and tactile feedback from the EMG activity
8 of the muscles involved in the execution of the phantom movement itself.

9 The participants underwent a 16-day treatment which commenced two weeks after enrolment into the
10 study. Pain evaluation, quantitative sensory testing (QST), and functional magnetic resonance imaging (fMRI)
11 were performed before and after training. Pain evaluation was again carried out at a follow-up six weeks after
12 the end of the training. A detailed daily pain diary was used to assess intensity and fluctuation of pain during
13 the entire duration of the study, which lasted 76 days in total.

14 It was anticipated that training the residual limb muscles, that are meant to move the phantom limb,
15 might represent an effective approach to improve motor control over the phantom, as concurrent information
16 about the intensity and distribution of the muscular activity meant to control the actual movement is returned.
17 The phantom-limb motor commands were fed back as visual and tactile information substitution with the
18 intent of providing a concurrent feedback of the volitional motor intention over the phantom limb movement
19 control. The administered multimodal (visual and tactile) feedback were used as an alternative sensory
20 information over the missing visual and proprioceptive information. As phantom movements involve motor
21 execution and a specific effort in contracting the residual limb muscles, in this study visualisation and tactile
22 sensations of such effort are meant to improve phantom motor control and provide synchronised feedback of
23 putative phantom movement. The multimodal visual and tactile feedback served even as reinforcement of the
24 sensory afferences already available at the stump level (proprioceptive afferences from muscular activity).
25 Functional magnetic resonance imaging (fMRI) mapping the tactile lip, lower arm, and (phantom) hand

1 representation was performed contra- and ipsilaterally before and after the three-week training, in order to
2 assess whether the training induced somatosensory cortical plasticity, ideally reducing potential maladaptive
3 plasticity.

4

5 **Materials and Methods**

6 The present study aimed to explore the treatment of PLP using a novel paradigm of training phantom-
7 limb movement control using stump muscular activity as a source of visual and tactile feedback. The
8 reduction of PLP intensity across the time of the treatment, at the end of it and at the follow up period of 6
9 weeks represented the main outcome of the study.

10 *Participants*

11 A group of 10 participants (five women, aged 57.7 ± 12.4 years, range: 28 – 75 years) were selected
12 from an initial screening interview. Inclusion criteria were: (i) major unilateral upper-limb amputation, (ii)
13 PLP at least twice a week, with an average peak intensity of 3 on a VAS scale (Visual Analog Scale anchored
14 with 2 points: 0 = no pain – 10 = the worst pain ever felt), (iii) amputation executed more than two years ago
15 from the enrolment, to rule out acute PLP. The average time since amputation was 17.7 ± 16.0 years (range: 6
16 – 52 years). All participants reported feeling a phantom limb.

17 Table 1 provides basic information about the participants, while Table S1 (Supplementary Material)
18 provides detailed information, including cause of amputation, pain medication and PLP frequency.
19 Participants were instructed to refrain from new pain therapies across the entire duration of the study. The
20 study was approved by the ethical committee of the University Medical Center Göttingen, Germany and
21 performed in accordance with the Declaration of Helsinki. Informed written and oral consent was obtained
22 from all participants prior to the intervention.

Participants	1	2	3	4	5	6	7	8	9	10	Mean (SD)
Gender	M	F	F	M	F	M	F	M	F	M	5M, 5F
Age (years)	59	56	59	28	60	64	64	75	63	49	57.7 (12.4)
Amputation side	L	R	L	L	L	R	L	L	L	L	2R, 8L
Amputation level	TR	TR	TR	TH	TR	TR	TR	TH	TH	TH	6TR, 4TH
Time since amputation (years)	6.2	14.7	5.7	13.8	6.2	7.2	8.2	52.9	26.2	36.7	17.7 (16)
Telescoping	N	N	N	N	N	Y	Y	N	Y	Y	4Y, 6N
Myoprosthesis user	Y	Y	Y	N	Y	Y	Y	Cos	N	Y	7Y, 3N
Pain intensity @ T1 (VAS)	6.5	8.5	3	4.3	3.5	6	4.1	7	4	4	5.1 (1.8)
Stump pain	N	Y	N	Y	N	N	N	N	N	N	2Y, 8N

Table 1. Participant details. “Telescoping” refers to a phenomenon in which the phantom limb of an amputee is not perceived at the location previously occupied by the intact limb but retracted inside the stump.

Further abbreviations: TR = Transradial, TH = Transhumeral, Cos = Cosmetic Prosthesis, VAS = Visual analogue scale.

Temporal succession

After a preliminary telephone interview to evaluate the participants’ eligibility according to the study inclusion criteria, the participants were enrolled. From two weeks prior to the start of the training (T0, Table 2) until the follow-up evaluation six weeks after the end of the treatment (T3) they were asked to complete a daily pain diary (Table 2), which consisted of an evaluation of PLP and stump pain intensity reported on a VAS scale and had to be completed four times per day. Also the characteristics of the pain and the pharmacological regimen were self-reported. Additionally, the PLP and stump pain domains were assessed in three sessions (pre, post, and follow-up evaluation at T1, T2, and T3, respectively) as reported in Table 2. Between T1 and T2, each participant trained, twice per day, five days per week for a total of 12 effective training days (within 16 days), with the protocol described below.

Time-point	T0		T1		T2		T3
Duration	1 day	2 weeks	1 day	16 days	1 day	6 weeks	1 day
Phase	enrollment		pre evaluation	training	post evaluation		follow-up evaluation
Tasks		daily pain diary completion	baseline and pain questionnaires, QST, fMRI	training 1hour, twice per day, 5 days/week, daily pain diary completion	pain and satisfaction questionnaires, QST, fMRI	daily pain diary completion	pain questionnaires

Table 2. Study trial timeline. Phases and time-points characterizing the progression of the study which lasted 76 days in total.

Baseline questionnaires = MPI-D, PCS, DASS-21, SF-36, PainDETECT, CEQ.

Pain questionnaires = VAS, SES.

QST = Quantitative Sensory Tests.

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3 *Phantom-limb treatment protocol*

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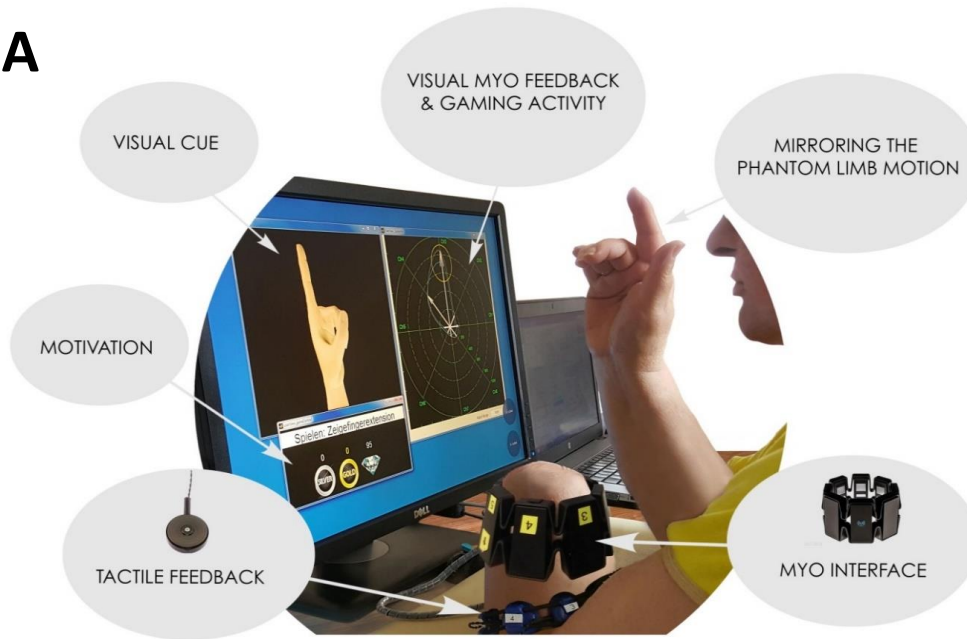
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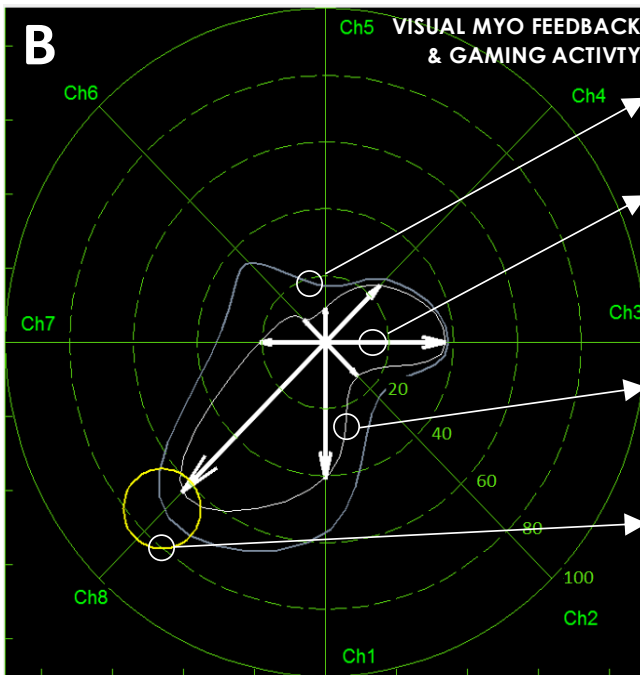
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The muscular activation at the stump level was recorded by eight superficial bipolar electrodes and differential electromyography (EMG) amplifiers with wireless data transmission (Myo™ Armband, Thalmic Labs Inc., Canada - Fig. 1A), evenly embedded in an elastic plastic band. The EMG band position was selected to obtain the best muscular activation (visually evaluated) across the eight acquisition locations. Sensory feedback was implemented by eight micro-vibrators (C3 Tactor, Engineering Acoustics Inc., USA) for tactile stimulation and an intuitive visual representation as visual feedback on a screen. Both of the feedback modalities conveyed the information of the eight EMG electrodes. The micro-vibrators were aligned and evenly positioned around the stump with an elastic band as each EMG amplifier provided the control signal for the corresponding vibrator. The position of the EMG amplifiers and micro-vibrators were marked with a medical skin marker to assure consistent positioning along the entire training period. Visually, the analysed EMG data were displayed using a radial plot arrangement to be congruent with the anatomical position of the acquired muscles (see Fig. 1A).

A



B



Reference Polar Plot:

Polar Plot of the MVC EMG

EMG activity: actual RMS EMG amplitude for each of the 8 amplifiers

EMG Polar Plot: Polar Plot of the actual EMG activity

Moving circle: moving target locked on the most active MVC EMG channel

Figure 1. Experimental set-up of the multimodal sensory-motor training.

A. All the main components of the system are reported. The visual patient interface is based on several windows which are used as a reference on the movement to be executed with the phantom limb: 3D-hand model: "Visual Cue"; window where scores are reported: "Motivation"; polar plot where visual feedback of myoelectric stump activity is reported: "Visual Myo Feedback". The "Myo Interface" is represented by the Myo™ Armband. The "Tactile Feedback" is generated using an elastic band with 8 embedded micro-vibrators.

B. Snapshot of the Visual Myo Feedback interface and Gaming activity. The polar space contains the axis to show the acquired EMG amplitude in % of the MVC (from Ch1 to Ch8). The patient interface shows in real-time i. the actual EMG activity acquired at each of the 8 amplifiers (8 white arrows: "EMG activity"); ii. the interpolated plot ("EMG Polar Plot") across the 8 RMS EMG values; iii. the interpolated polar plot acquired during the calibration phase and used as a reference for the participants ("Reference Polar Plot"); iv. the "Moving circle" used as a target for the gaming activity. The Moving circle is locked on the most active channel (Ch8 in this example) of the corresponding MVC calibration acquisition and it moves along this channel promoting a contraction and release of the stump muscles as the participant has to keep the EMG activity inside it and close to the Reference Polar Plot to score (see text for further details).

The EMG data were sampled at 200 Hz, acquired through a Bluetooth communication protocol, rectified, and smoothed via RMS (moving windows of 250 ms), displayed on the polar plot (Fig. 1B) and used to command, via USB port, the controller of the micro-vibrators. The training software, composed of a participant interface and an operator interface, was developed in Matlab (MathWorks, USA). The motor activity was based on a game-like exercise to train the execution of a selection of phantom movements among a list of ten movements, including five wrist movements (wrist flexion/extension and pronation/supination, ulnar deviation), three hand movements (hand open, key grip, fine pinch) and two finger movements (index-finger extension, ring-finger flexion).

Each training session started with calibration of the training software and lasted around one hour. The operator selected a series of phantom movements to train during the calibration phase, taking into account the actual current control ability of the participant. The calibration consisted of a slow maximal voluntary contraction (MVC) of the phantom movements to be trained, played in a sequential way. The contractions were supposed to be performed by following the visual cues provided by a 3D hand model on the participant interface (Fig. 1A – "visual cue"). This procedure was necessary to normalise the visualisation of the EMG

1 data and to store the polar plot shape of the MVC EMG amplitude for each calibrated movement (See Fig. 1B
2 – “Reference Polar Plot”). Scaling the EMG individually, based on the participants’ specific MVC values,
3 provided a clear visual feedback in all the trained phantom movements as the EMG values moved within the 0
4 – 100% MVC range.

5 Only those movements selected during the calibration phase were trained during the following training
6 phase. During each required phantom-movement run, the name of the movement to be realised (e.g. wrist
7 supination) was indicated. The participants executed the prescribed phantom-limb movement starting from
8 and returning to a neutral position of the limb (elbow 90° flexed, wrist in neutral position), following the
9 motions of the 3D hand model. The 3D hand model informed the participants about the type, way and speed
10 of phantom movement to be executed in each trial. Each prescribed movement therefore implied a
11 “contraction phase” in which an increasing EMG amplitude was registered and a “release phase” where the
12 EMG amplitude decreased until the resting level. The 3D hand model motion was synchronised with the
13 contraction and release phase prompted by the Reference Polar Plot, which expanded and collapsed showing
14 the participants the amount (in % of MVC) of EMG activity to be generated during the prescribed phantom
15 movement to train (see a snapshot of this activity in Fig. 1B). While the hand model and the actual polar plots
16 (Fig. 1B – “EMG Polar Plot” and “EMG activity”) gave the participant visual feedback about how to properly
17 control the stump muscular activity, the vibrators tactilely reinforced the mechanoreceptive feedback of the
18 activated muscles. They were proportionally controlled modulating the vibration amplitude using, in real time,
19 the amplitude of the analysed EMG data (Fig. 1B – “EMG activity”). Each vibrator delivered a stimulus
20 intensity proportionally modulated with the intensity of the normalized EMG amplitude expressed at the
21 corresponding amplifier, with a vibration frequency of 100 Hz.

22 During the training runs, the participants were additionally guided in controlling the EMG amplitude
23 via a reference plot created using the MVC values registered in the previous calibration phase (Fig. 1A –
24 “visual myo feedback and gaming activity” and Fig. 1B – “Reference Polar Plot”). The gaming activity
25 provided a further reinforcement to improve the control of the phantom movement. A moving circle was

1 shown on the reference polar plot as a target for the participants, which they were asked to follow using the
2 EMG polar plot. The circle moved to and from the most active EMG channel recorded during the
3 corresponding calibration phase (Fig. 1B – “Moving circle”) locked on the reference polar plot. To be
4 successful on a run, they had to stay inside the circle for a certain amount of time, depending on the
5 determined difficulty of the training session. A scoring system, based on three different remunerations (a
6 silver coin, a gold coin, and a diamond), was devised to engage the participants and avoid frustration (Fig. 1A
7 – “motivation”). As further motor reinforcement, the participants were asked to mirror the phantom movement
8 with their intact side. If a participant could e.g. only marginally open the phantom hand, then he/she should
9 also only marginally open the intact hand. As soon as the selected movements were rather well executed,
10 another new phantom movement was added to the training.

11 The developed training software gave the operator the possibility of tailoring the training activity for
12 each of the patients, as every parameter was adjustable, e.g. control over the selected movements, the
13 movement speed, which had a smooth sinusoidal profile, its amplitude in terms of EMG range of activity (as a
14 percentage of MVC), the amplitude of the target circle, and the “polar plot in-circle” time duration for scoring
15 was provided. These parameters were selected and changed according to the single participant’s abilities and
16 improvements to obtain challenging and still manageable training sessions.

17 *Baseline and pain questionnaires*

18 Clinical characteristics, exploration of painful and non-painful phantom phenomena and stump
19 sensations were obtained with the questionnaire developed by Kern and colleagues (39) at the pre-evaluation
20 session (T1, Table 2). During the same session, the participant’s treatment expectancy and rationale credibility
21 were measured with the CEQ (40) as factors possibly representing non-specific treatment effects (41). PLP
22 and stump pain domains were evaluated using the following questionnaires: (i) the West Haven-Yale
23 Multidimensional Pain Inventory (MPI-D) (42, 43), a reliable and valid measure of physical functioning of the
24 pain domain; (ii) the pain perception scale (“Schmerzempfindungsskala”, SES) to measure the affective and
25 sensory characterization (44); (iii) the PainDETECT screening questionnaire to identify neuropathic pain
26 components (45); (iv) the Pain Catastrophizing Scale (PCS) as a measure of catastrophic thinking related to

pain (46) as it can be a risk factor for chronicity (47, 48); (v) the Depression, Anxiety, Stress Scales (DASS-21), as a self-rating measure of depression, anxiety, and stress (49); and (vi) the 36-Item Short Form Health Survey (SF-36), as a measure of the general health status of the participants (50). Additionally, at T2 a satisfaction scale anchored with 2 points (0 = completely dissatisfied, 100 = completely satisfied) was used to rate the participants' satisfaction with the received training.

Quantitative Sensory Tests (QST) and two-point discrimination test

During the pre-evaluation (T1, Table 2), a stump mapping procedure was performed. Systematic touch was applied to the distal portion of the stump in order to determine any points giving rise to referred sensations in specific parts of the phantom hand or fingers. The point triggering the strongest referred sensation of a finger was then marked on the stump and used as one of the location for the QST and the two-point discrimination test (see QST Supplementary Material). In the few participants where several finger sensations could be elicited, one of the most sensitive spots at the stump was used as the “stump trigger point”.

Two-point discrimination test was administered to evaluate the participants' somatosensory acuity (51). The two-point discrimination test measures the participants' ability to perceive two stimuli simultaneously presented at varying distances from each other as distinct. The minimal distance where the stimulations are separated is given in mm. It was measured using two rounded tips of a sliding calliper, applying just the weight of the calliper. Starting at 2 cm, the sliding-tips distance was reduced by 1 mm each trial, or increased by 1 mm over the location with lower sensibility (on the stump and the shoulder), until the participant could no longer feel a separation between the two points, or start feeling the two tips as separated stimuli. The two-point discrimination test was repeated three times, data were presented as mean value (52). The evaluators administering the baseline interview, the pain questionnaires, the QST and the two-point discrimination test did not take part in the training process to avoid a possible source of bias.

2 We assessed the possible cortical reorganisation with fMRI. Functional and anatomical magnetic
3 resonance imaging was performed using a 3 T Tim Trio scanner (Siemens, Germany) and standard imaging
4 sequences at time points T1 and T2 (Table 2). Standard anatomical (1x1x1 mm³) and functional
5 measurements (2 x 2 x 2 mm³, 10% gap, repetition time = 0.8 s) were performed, as described in Table 3.
6 During the functional measurements, the skin areas to be explored were stimulated using a traditional brush or
7 an air-puff stimulator developed for utilisation in the scanner environment (pneumatic device), in order to
8 evoke cortical activation in the primary sensory motor cortex.

Run 0	Anatomical image of the brain
Run 1	Lips (air-puff) & “digit” (stump location with digit sensation, brush)
Run 2	Arm (stump location without digit sensation, brush)
Run 3	Lips (air-puff) & intact-site digit (corresponding to Run 1, brush)
Run 4	Arm (location on intact side corresponding to stump location of Run 2, brush)

9
10 **Table 3. Sequence of (f)MRI measurements.** Sequence of acquisitions comprising anatomical MRI and fMRI with different stimulation sites.

11
12 In the first run (Run 1, Table 3), the lips were stimulated via air-puff stimulation, and a stump spot was
13 brushed by the experimenter (using a block paradigm with two conditions, alternating with rest periods), with
14 the stump location being the location with the strongest referred phantom finger sensation (selected during the
15 QST, see corresponding Methods section). In the second run, a slightly more proximal location (at the
16 forearm/upper arm in transradial/transhumeral amputees, respectively) was brushed in a rest/stimulation block
17 design. In the third run, the lips and the intact digit corresponding to the amputated one in Run 1 (commonly
18 the thumb or index finger) were stimulated, while the fourth run served for mapping the location at the intact
19 forearm/upper arm that corresponded to the arm position in Run 2.

1 *Training data*

2 The number of trained phantom movements, number of repetitions, single movement time duration
3 and tracking error were acquired for each daily session through the entire training period. The tracking error
4 was calculated as the sum of the absolute difference between the actual RMS EMG activity (Fig. 1B) and the
5 reference polar plot (Fig. 1B) created using the MVC values registered during the calibration phase. The mean
6 value of the tracking error was calculated for each executed trial.

7 *Data analysis and statistics*

8 The acquired EMG data were exported and processed offline in Matlab (MathWorks) and the data and
9 scores from the pain-domain evaluation and QST tests were noted on Excel spreadsheets (Microsoft). All data
10 were reported as mean values \pm standard deviation (SD), or standard error (SE) of the mean when indicated.
11 The training data were clustered in 3 groups composed of 4 consecutive training days, from day 1 to day 4 as
12 the initial training period, from day 5 to 8 as mid training and 9 to 12 as the last training period. Average and
13 standard deviation of the three training periods were analysed. Where data distributions were not Gaussian
14 (according to Shapiro-Wilk tests), statistical evaluations were performed using the Wilcoxon Signed Rank test
15 for paired data or the Mann-Whitney U test for unpaired data. Repeated measures ANOVA and Student's t
16 test for independent samples were used for normally-distributed data. Bonferroni correction was applied for
17 multiple comparisons. Significance was considered when $p < 0.05$. Pearson r (ESr) were used to estimate the
18 effect size for normally distributed data. ESr less than 0.19 was classified as “very weak”, 0.2 – 0.39 as
19 “weak”, 0.4 – 0.59 as “moderate”, 0.6 – 0.79 as “strong”, 0.8 – 1 as “very strong” (53). The 95% confidence
20 interval (CI) for the mean difference was calculated. Statistical analysis was performed with the SPSS
21 Statistics software (IBM, Version 22).

22 Using the PLP intensity between T1 and T2 (Fig. 2), an post-hoc analysis of the achieved power of the
23 study was executed with the Software G*Power (version 3.1.9.2) (54) retrieving a power, $1-\beta$ error
24 probability, of 0.99 (N = 10, type I error probability $\alpha = 0.05$, Cohen's dz effect size = 1.73 (55))

fMRI analysis was performed in Brain Voyager (Brain Innovation, Maastricht, The Netherlands).

Using the general linear model, cortical representation areas (statistical maps) for each of the stimulated sites could be statistically defined. For each individual, the cortical mesh was reconstructed for each hemisphere, and the statistical maps were projected onto it. As such, the statistically most significant vertices (peak vertices) and their 3D coordinates could be determined for each stimulated site. Differences in these peak locations between the representations ipsi- and contralateral to the amputation could be assessed. As this procedure was performed both pre- and post-training, possible shifts after training would be recognisable. Three Wilcoxon Signed-Rank tests were performed for each of the three 3D axes, in which the respective coordinates of the average-lips peak-activations were compared 1) prior to the training between hemispheres and pre-to-post-training within the hemisphere 2) contra- and 3) ipsilateral to the amputation.

Results

Baseline pain characteristics

The patients' baseline pain characteristics, assessed with the Pain Inventory domains (MPI-D), were compared to normative values derived from a population of 185 patients with chronic pain (43). Participants reported a significantly lower amount of interference that pain had on their life (1.78 ± 1.11 (mean \pm SD), $t(193) = 2.38$, $p = 0.0179$, $0.27 \leq CI \leq 1.69$, normative – participants' mean), a lower level of distress caused by pain (2.29 ± 0.85 , $t(193) = 3.19$, $p = 0.0016$, $0.7 \leq CI \leq 1.82$), a higher perception of life-control (4.65 ± 0.81 , $t(193) = -2.17$, $p = 0.0308$, $-1.38 \leq CI \leq -0.31$), a higher performance of household chores (3.91 ± 1.5 , $t(193) = -4.99$, $p < 0.0001$, $-3.1 \leq CI \leq -1.19$), considered as one of the common everyday life activities, and a higher performance on the general activity subscale (sum of all the subscales of the activity domain, 3.52 ± 0.61 , $t(193) = -3.07$, $p = 0.0024$, $-1.31 \leq CI \leq -0.5$).

The entire group of participants, with one exception, reported a catastrophizing score less than 30, which is the cut-off value for clinical relevance (56-59). The PCS score of 38 obtained for one patient is equivalent to the 90th percentile of the normative clinical distribution. The same participant showed an

1 emotional state of depression at a moderate level (Depression, Anxiety, Stress Scales (DASS-21), Depression
2 Subscale = 16) while two participants reported a state of mild anxiety (DASS-21, Anxiety Subscale = 8) (49).
3 The average physical functioning score of the 36-Item Short Form Health Survey (SF-36) was 77.5 ± 29.2
4 (mean \pm SD), revealing no significant difference ($t(2479) = -0.79$, $p = 0.428$, $-25.07 \leq CI \leq 11.29$) to
5 normative healthy-participant data ($N = 2741$, 70.6 ± 27.4) (60-62). The bodily pain score of the SF-36
6 showed a significant difference compared to normative data (53.75 ± 20.21 , $t(2479) = 2.11$, $p = 0.0349$, $4.44 \leq$
7 $CI \leq 29.59$).

8 The PainDETECT screening questionnaire score revealed an unlikeliness of a neuropathic component
9 of PLP in six of the participants since their score was lower than 12, being the cut-off point for neuropathic
10 pain. No clear indications could be determined (45, 63) for the other four participants since their
11 PainDETECT scores fell between 12 and 19. For these participants the screening results were ambiguous, a
12 neuropathic pain component could be present.

13 *Treatment effect and retention on PLP intensity*

14 PLP intensity was obtained by averaging the scores from the two VAS scales (Visual Analog Scale
15 anchored with 2 points: 0 = no pain – 10 = the worst pain ever felt) used to rate the intensity of the actual pain
16 and the average pain over the last week. A significant reduction in pain intensity was observed from T1 to T2
17 (from 5.08 ± 1.79 to 4.02 ± 1.7 , mean \pm SD, $F(2,8) = 19.36$, $p = 0.00114$, $0.49 \leq CI \leq 1.62$, $ESr = 0.94$, $SS(W)$
18 $= 12.29$, sum of squares within subjects) and T1 to T3 (3.55 ± 1.8 , $p = 0.00101$, $0.7 \leq CI \leq 2.3$, $ESr = 0.89$),
19 reaching the clinically significant reduction threshold of more than 30% (32.1%, with 6 participants showing a
20 decrease of more than 30% in PLP intensity and 5, an approximate decrease of two points on the VAS scale)
21 at T3 (64). No significant difference was found between T2 and T3 ($p = 0.317$, $-0.2 \leq CI \leq 1.23$, $ESr = 0.89$)
22 (Fig. 2). The multimodal sensory-motor training was therefore clinically effective in determining a reduction
23 of PLP intensity which lasted for at least six weeks.

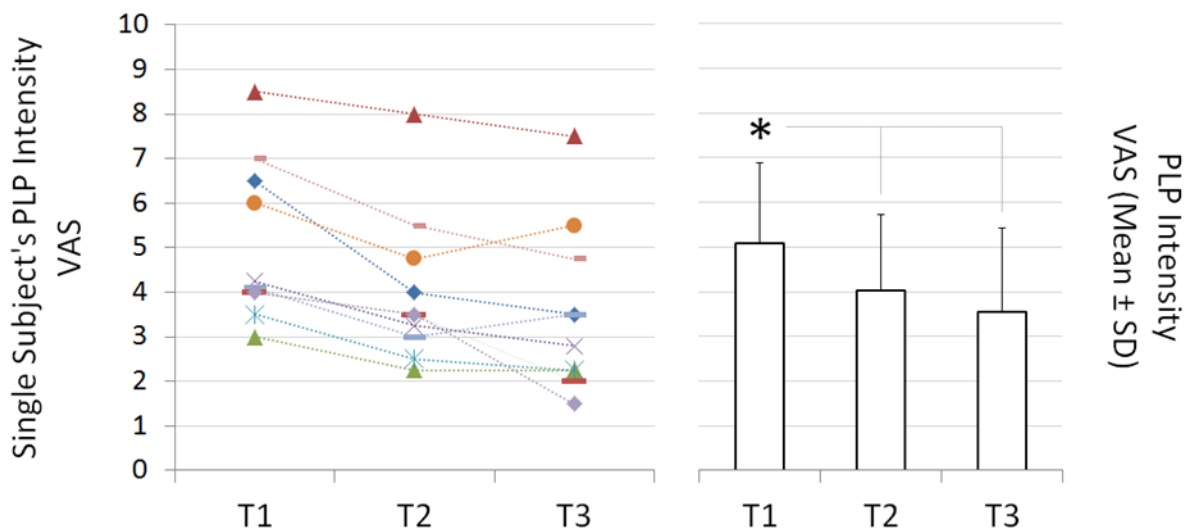


Figure 2 Pain intensity: Treatment and retention according to the assessments at T1, T2, and T3. The graph on the left reports the individual evaluation of PLP intensity as the average of the actual pain and average pain over the last week. The graph on the right reports the mean and standard deviation of pain intensity scores for the entire group showing a statistically significant reduction ($p < 0.01$, reported with an asterisk) of PLP intensity between the evaluation executed before treatment (T1) to immediately after (T2) and six weeks after (T3) treatment.

As a secondary outcome, the affective and sensory characterization of pain was obtained using the pain perception scale (“Schmerzempfindungsskala”, SES) (44). SES revealed that the affective characterization of PLP reduced significantly after the training period, from 27.8 ± 9.8 (mean \pm SD) at T1 to 21.9 ± 8.5 at T2 ($F(2,8) = 6.01$, $p = 0.033$, $0.44 \leq CI \leq 11.35$, $ESr = 0.8$, $SS(W) = 182.86$), but was not significantly different from T1 at T3 (23.6 ± 6.3 , $p = 0.204$, $-1.7 \leq CI \leq 9.9$, $ESr = 0.78$). No statistically significant difference was found for the same subscale score between T2 and T3 ($p = 1$, $-9.4 \leq CI \leq 5.8$, $ESr = 0.41$), showing that the improvement in the affective characterization of PLP lasted just after the end of the treatment. No differences were reported for the sensory characterization of PLP, a second subscale score of the SES. Hence, the training reduced the significance of the pain (seen as less cruel, horrible or violent) but not its characteristics regarding sensory perception (temperature or rhythmicity). At T2, a questionnaire rating the patient’s satisfaction with the received intervention showed a high treatment satisfaction ($94.1\% \pm 8\%$, mean \pm SD). Table 4 visually displays the data reported above on the VAS to measure the PLP intensity and the SES results across the evaluation phases of the study.

Time-point	T1	T2	T3
Phase	pre evaluation	post evaluation	follow-up evaluation
PLP Intensity VAS (Mean \pm SD)	5.08 \pm 1.79	4.02 \pm 1.7 **	3.55 \pm 1.8 **
SES Affective Characterization (Mean \pm SD)	27.8 \pm 9.8	21.9 \pm 8.5 *	23.6 \pm 6.3
SES Sensory Characterization (Mean \pm SD)	17.1 \pm 5.1	16.0 \pm 5.0	16.6 \pm 5.3

Table 4. Treatment effect on PLP. Averaged VAS values (Visual Analog Scale anchored with 2 points: 0 = no pain – 10 = the worst pain ever felt) to rate the intensity of the PLP across the three evaluation phases (at T1, T2 and T3). SES Affective and Sensory characterization is reported for the same phases. The statistics is referred to the comparison between the values at T1 and T2, or T3.

* = $p < 0.05$

** = $p < 0.01$

SES = Schmerzempfindungsskala

Treatment effect and retention according to the pain diary

Each participant was instructed to complete a daily pain diary where the PLP intensity was reported four times per day: in the early morning, at midday/lunchtime, in the afternoon, and at night/before sleeping. The participants were instructed to rate their PLP intensity using a VAS scale taking into account also the intensity and duration of pain attacks (if any). The completion of the pain diary commenced two weeks before treatment (T0) and ended at the follow-up (T3).

The PLP intensity was extracted from the pain diaries until six weeks post-training to assess the retention of the induced positive effects. We did not include T1 and T2 in the evaluation, since during these two days the participants underwent long and possibly fatiguing evaluations (QST and functional Magnetic Resonance Imaging) which could have affected their pain (65). The PLP intensity reported by the participants for one week before entering the intervention were averaged and used as a reference. From T1 to T3 (excluding the day of T2) the data from all the participants were pooled in groups of two consecutive days and compared to the reference. The resulting two-day pooled PLP intensity data showed statistically significant differences ($p < 0.047$) compared with the reference value (black diamond, Fig. 3), at 5th and 8th comparison

1 (last two days of treatment) until the 23rd comparison, with the exclusion of the 17th and 20th (see Fig. 3).

2 Thus the effects of the treatment lasted for 30 days.

3 To analyse the daily variability of PLP intensity, the standard deviation of the four daily scores of each
4 patient was used following the same pooling approach reported above for the average PLP intensity.

5 Significant reductions of the daily variability of PLP just at the 9th, 10th and 12th comparisons ($p < 0.029$) and
6 after the 20th with a sparse occurrence were found (see Fig. 3). Hence, an effect of reduction in daily PLP
7 variability was obtained, which lasted until 8 days after the end of the intervention, but with a less continuous
8 characteristic than the mean PLP intensity reduction. The reduction in daily PLP variability demonstrates that
9 the oscillatory characteristics of PLP were significantly reduced.

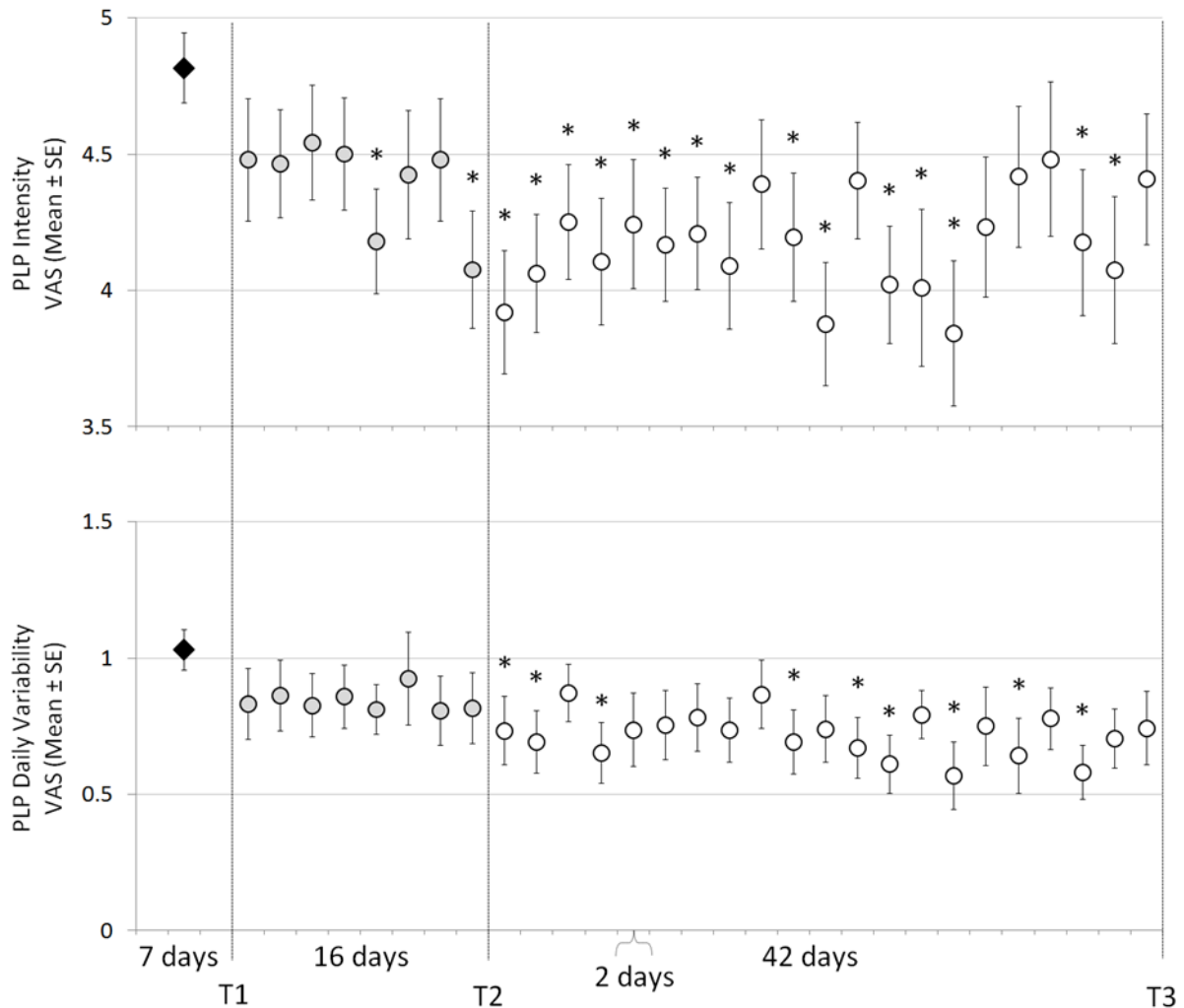


Figure 3 Treatment and retention according to the pain diary. The upper graph reports the average PLP intensity of the two-day pooled data from all participants for their daily PLP evaluation for 7 days before starting the treatment (black diamond before T1), each two consecutive days during treatment (grey circles between T1 and T2), and each two consecutive days after treatment until follow-up (white circles between T2 and T3). The lower graph reports the average standard deviation of the pooled data for the daily PLP evaluation from all participants during the same time frames as reported above.

Statistics were calculated comparing to the reference value (black diamond before T1) with each single following average (grey and white circles from T1 to T3), significant differences are marked by an asterisk ($p < 0.047$).

Muscular activity of the residual limb and correlation with PLP intensity

Across the 12 effective days of training (16 in total, considering the weekends when the participants received no training sessions) no differences were registered regarding the number of trained phantom movements (hand and wrist movements). The participants were able to execute and train on 7.40 ± 2.57 (Mean \pm SD) types of movements across the first 4 days to end with 8.00 ± 1.98 different movements' types during the last 4 days of training. No significant differences ($p > 0.1$) were found across the three clustered groups of data (days 1-4 as the initial training period, 5-8 mid training and 9-12 last training period). On the contrary, the number of phantom movements' repetitions executed on average (\pm SD) across the three training periods showed a significant increase ($p < 0.001$) moving from 105.43 ± 45.22 , during the initial training period (days 1-4) to the last training period (days 9-12) when the participants were able to repeat 176.97 ± 69.00 the prescribed phantom movements (148.77 ± 52.02 average repetitions during the mid training period, days 5-8). If averaged across each single day, the number of phantom movements' repetitions negatively correlate with the reduction in PLP intensity acquired with the pain diary, with Pearson's $r = -0.68$ ($p = 0.013$). The tracking error, as percentage of the EMG MVC value registered during the calibration, indicates the ability to control the effort of the residual limb muscles to move the phantom limb. The tracking error represents the distance between the actual EMG activity to execute the prescribed phantom movement to train (See Fig. 1B, EMG Polar Plot) and the reference EMG polar plot to follow (Fig. 1B, Reference Polar Plot). The tracking error showed a significant reduction from the initial compared to the last training period ($p <$

0.001, from 126.91 ± 77.90 %MVC to 108.21 ± 69.58 %MVC, respectively). The average \pm SD of the tracking error during the mid training period was equal to 115.83 ± 80.97 %MVC. Interestingly, the daily average of the tracking error values, across the 12 days of effective training, was positively correlated with the reduced PLP intensity with Pearson's $r = 0.65$ ($p = 0.022$). A significant reduction between the first and last training period was achieved for the single trial duration time (s), and therefore, a consistent increase in the speed of the executed phantom limb movements ($p < 0.001$, from 22.01 ± 4.38 s to 16.36 ± 8.14 s, respectively; 17.94 ± 7.29 s for the mid training period). A positive correlation was reached between the daily average of the single trial duration time and the PLP intensity, with Pearson's $r = 0.68$ ($p = 0.014$).

Placebo effect and expectancy

Expectation, hope, motivation, therapeutic relationships, conditioned responses and other psychological processes of the patient can contribute to a placebo effect (66). Among them, expectancy is one of the most influential components. The role of expectancy, where the placebo effect generates from the anticipation that a treatment will end in a specific outcome, has been verified by a systematic review of 85 studies (67). The Credibility and Expectancy Questionnaire (CEQ) was administered in this study to report the level of bias regarding the efficacy of the treatment induced by expectation as a placebo effect (40). The results obtained from the CEQ, divided in "credibility" and "expectancy" scores ($49.6 \pm 31.9\%$, $35.1 \pm 19.4\%$, respectively, mean \pm SD), showed no significant correlation with the reduction of PLP intensity (Fig. 2) between T1 and T2 (expressed as percentage of the value reported at T1), with Pearson's $r = 0.408$ ($p = 0.242$) for credibility and 0.266 ($p = 0.458$) for expectancy. These results indicate that the PLP-treatment effects are unlikely to be attributed to a placebo effect.

QST and two-point discrimination test

A complete series of QST were executed at T1 and T2 to report any training effects on the peripheral components of the aetiology of PLP (e.g. impaired sensory perception). Following the recommendations of Rolke et al. (68), log-transformation with base 10 to achieve normal distribution was executed for the following QST variables: cold and warm detection thresholds (CDT, WDT), thermal sensory limen (TSL),

mechanical detection threshold (MDT), mechanical pain threshold (MPT), vibration detection threshold (VDT) and pressure pain thresholds (PPT) (see QST supplementary material for further indications). The QST was performed at four locations: (i) at the stump trigger point for a phantom thumb/finger, (ii) at the corresponding thumb/finger of the intact side, (iii) on the lateral aspect of the shoulder on the amputated side, (iv) at the equivalent location on the intact side (see the corresponding Methods section for further details). Statistical comparisons were executed between T1 and T2 for the same location. For the Cold and Heat Pain Threshold (CPT and HPT, $N = 8$, two participants were removed from the analysis as they reached the limit of the Thermal Analyser without reporting pain), a statistically significant difference was obtained between T1 and T2 for the stimulation at the stump trigger point (i), with a threshold change from $12.5 \pm 10.2\text{ C}^\circ$ to $16.2 \pm 8.2\text{ C}^\circ$ (mean \pm SD, $p = 0.00739$, $1.34 \leq CI \leq 6.03$, ESr = 0.97) for CPT and from $44.8 \pm 3.6\text{ C}^\circ$ to $42 \pm 3.9\text{ C}^\circ$ ($p < 0.0001$, $1.91 \leq CI \leq 3.52$, ESr = 0.97) for HPT. A similar change was observed on the lateral aspect of the shoulder on the amputated side (iii) (CPT from $13.6 \pm 9.3\text{ C}^\circ$ to $19.1 \pm 8.4\text{ C}^\circ$, $p = 0.00851$, $1.91 \leq CI \leq 9.12$, ESr = 0.88, HPT from $45.3 \pm 3.5\text{ C}^\circ$ to $41.7 \pm 2.9\text{ C}^\circ$, $p = 0.00118$, $2.25 \leq CI \leq 4.83$, ESr = 0.89).

For the two-point discrimination test a significant difference from T1 to T2 was found only at the stump trigger point (i), indicating an improvement in tactile discrimination ability at the stump level (from $45.3 \pm 16.5\text{ mm}$ at T1 to $28.4 \pm 19.4\text{ mm}$ at T2, $p = 0.01209$, $4.99 \leq CI \leq 28.75$, ESr = 0.69, $N = 8$, same participants used as to report CPT and HPT). No other statistically significant differences were observed.

fMRI data analysis

During fMRI, somatosensory stimulation was performed at a phantom digit and another stump location, at the respective finger and arm position on the intact side as well as at the lips. Significant cortical activation could be observed upon that stimulation for each of these skin locations and each participant (except for participant 7, who refused to undergo fMRI, and participant 4, who did not show any significant activation in the primary sensorimotor cortex for some stimulated spots, making a between-spots analysis impossible, and therefore was excluded) in the hemispheres ipsi- and contralateral to the amputation. As the average Euclidean distance between the peak lip activations of the two runs within a session was only $3.8 \pm$

1 1.2 mm (less than two voxels), rather reproducible statistical maps were obtained, indicating a high imaging
2 quality. The activation pattern can be seen in a representative participant in Figure 4A and in the 4D peak-
3 activation coordinates given in Figure 4B. Prior to training, contralateral to the intact side the lips were
4 represented most lateral, anterior, and inferior, followed by the digit and then the arm representation, as
5 expected from the sensory homunculus by Penfield and Rasmussen (69-71). While the lips-arm arrangement
6 was the same contralateral to the amputation, the phantom “digit”, effectively being a stump area, was found
7 more medial, posterior, and superior compared to the respective digit representation of the intact side, not-
8 differentiable from the slightly more proximal arm area (not statistically tested). In contrast to our expectation
9 from previous literature (27, 36, 72, 73), the lip representations did not vary in position between the intact and
10 the amputated side pre training (p-values along all three coordinates > 0.5). Hence, the participants did not
11 seem to have undergone cortical plasticity, such that a training effect was very unlikely to be found.
12 Nevertheless looking for a training effect, the average lips 3D coordinates pre- and post-training were
13 compared within both hemispheres. While no significant training-induced changes were found for either of the
14 two hemispheres (Fig. 4), a trend with a large effect size ($p_{x\text{-axis}} = 0.06$) for a small lateral lips-representation
15 shift (1.4 ± 1.7 mm on average) was observed along the x-axis contralateral to the amputation, in accordance
16 with a reversal of maladaptive plasticity.

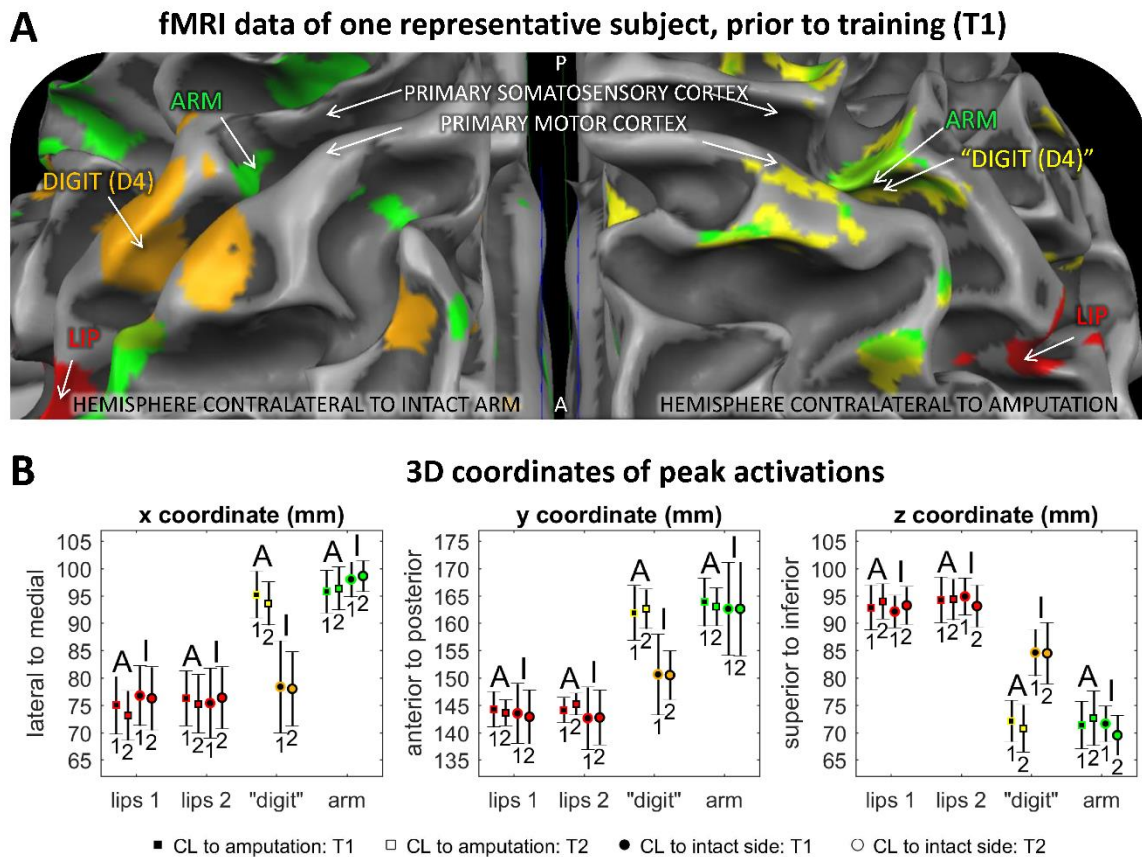


Figure 4: fMRI data. A) fMRI data of one representative participant, prior to training, shown on the reconstructed white-to-grey-matter surface. The sensory-evoked activations of the lips (red), intact digit (here the ring finger, D4, orange), phantom "digit" (perceived D4, yellow), and lower arm or stump (green) are marked. A = anterior, P = posterior. **B)** 3D coordinates of fMRI activations across participants. For each axis, the mean and standard deviation of the fMRI representations contralateral (CL) to the amputated (squares, also marked with large A) and intact (circles, also marked with large I) side, obtained before (black-filled, also marked with 1 below) and after training (black-framed, also marked with 2 below), are given for the (twice-measured) lips 1 and lips 2 representations (red) as well as for the digit (orange) or phantom "digit" (yellow) and arm (green) representation.

Discussion

The multimodal sensory-motor training employed in this study led to a significant PLP-intensity reduction which was greater than 30%. The treatment was well accepted by the participants since on average they rated it with ~95% satisfactory. These significant treatment effects have been obtained irrespective of the large variability in patient characteristics, regarding the level of amputation (e.g. transhumeral and

1 transradial), stump pain, telescoping, and stump mapping. Mirror therapy is one of the most useful non-
2 pharmacological approaches for PLP treatment (20). In fact, a previous RCT study reported a reduction in
3 PLP intensity from approximately 30/100 initially to 5/100 after four weeks of mirror therapy (20, 28),
4 (relative PLP reduction of ~83.3%), with a further PLP reduction to approximately 3/100 after a further 4
5 weeks of treatment (8 weeks of treatment in total, total PLP reduction of ~90%). The administered therapy
6 lasted 15 minutes per day suggesting that mirror therapy is an effective approach in terms of therapy time.
7 Even if more effective, mirror therapy may be less effective when a telescoped phantom is perceived (30) and,
8 contrary to the proposed approach, cannot be applied to treat PLP in bilateral amputees. Whereas, the
9 proposed multimodal sensory-motor training tested in this study led to a significant PLP reduction both for the
10 four participants with and six participants without a telescoped phantom. Because of the high incidence of
11 telescoping (around one-third of amputees (74)), the results of this study are promising for a broad clinical
12 applicability of the approach. The training also reduced stump pain from 2.25 ± 1.7 to 0.87 ± 1.2 (mean \pm SD)
13 in the two participants who suffered from such pain.

14 The multimodal sensory-motor training adopted in this study represents a new approach for PLP
15 treatment, addressing phantom motor execution enriched with sensory feedback of the moving phantom arm.
16 Among the several strategies currently available for PLP treatment (34 commonly employed and 13 new
17 approaches specifically addressing maladaptive plasticity (27)), which have limited clinical effectiveness (27,
18 64, 75), the proposed multimodal sensory-motor training represents the first effective treatment using
19 multimodal sensory feedback (visual + tactile) of phantom motor execution. The presence of a multimodal
20 sensory feedback is fundamental, as vision alone has only led to a significant increase of phantom awareness
21 or control (75). The inclusion of additional sensory feedback differentiates our approach from another recently
22 proposed method (38) that provided a visual, EMG-controlled representation of the phantom limb movement
23 by exploiting machine learning. In the treatment proposed in the current study, tactile stimulation patterns
24 proportional to the stump muscles' level of activity provided a functional perceptual input aimed at improving
25 the volitional control over the motor output during phantom movements. The tactile stimulus during phantom
26 motor execution could have had an effect on (i) providing a further sensory feedback substitution (more than

1 just the visual input), (ii) potentially improving sensory discrimination on the stump as suggested by the
2 results of the two-point discrimination test. However, even if approaches aimed at improving sensory
3 discrimination at the stump have been shown to be effective methods for PLP reduction (35-37), the main aim
4 of the tactile stimulus, provided during our study, was to provide an additional feedback to the volitional
5 motor control of the phantom limb with no direct intention of improving sensory discrimination over the
6 residual limb as the two objectives can have different working mechanisms.

7 The positive effects of the treatment lasted at least until the end of the study. The PLP intensity was
8 significantly lower at T2 compared to prior to training (T1) and remained significantly lower six weeks after
9 the end of the treatment (follow-up evaluation at T3). The more detailed assessment of PLP intensity and its
10 daily fluctuations, obtained from the pain diary, showed that the reduction of PLP lasted for 30 days after the
11 end of the treatment. This difference can be explained by taking into account the different time frames used to
12 rate PLP. The PLP intensity accounts for actual pain and average pain over the previous week, whereas the
13 evaluation executed using the pain diary data is based on a day-by-day multiple rating. The pain diary also
14 allowed precise documentation of daily PLP fluctuations, which could indirectly estimate the troublesomeness
15 of the daily pain attacks (76). A significant simultaneous reduction of both the PLP intensity and the daily
16 PLP fluctuations and pain attacks has the potential to lead to an improved quality of life for the participants
17 (77). The results reported in Fig. 3 show a linear decrease in the average PLP intensity with the progression of
18 the treatment (data between T1 and T2, Spearman's $\rho = -0.669$, $p = 0.0485$), being significant at the last two
19 days of the treatment. It would be expected that placebo-biased participants would have shown a more rapid
20 pain reduction during the initial days of treatment (78). Moreover, the results of the CEQ, rating the
21 expectation and credibility biasing effects on the treatment to be received (40, 79, 80), makes it unlikely that
22 the effects obtained on PLP were determined only by a placebo effect. While the proposed method
23 successfully reduced PLP, no significant cortical shifts were observed between T1 and T2, expect for a trend
24 for a small lateral lips-representation shift. However, as no shift in the lips representations was found between
25 the intact and affected hemisphere in the included amputees even prior to training (at T1), in contrast to

1 several earlier studies (27, 36, 72, 73), no significant maladaptive plasticity was observed in the present study
2 anyway, making a change from T1 to T2 rather unlikely.

3 As described in the methods section, the lip was stimulated via air puffs, while a brush was used for
4 stimulation of the (phantom) digit and stump. The reason for this heterogeneous stimulation was the
5 following: while manual brush stimulation proved to be difficult to control and uncomfortable at the lips, pilot
6 studies revealed a too weak activation at the stump upon air-puff stimulation, making the use of a brush
7 necessary at that site. Nevertheless, we are confident that this heterogeneity has not prevented us from
8 observing cortical plasticity, as the comparison between locations stimulated with different methods was not
9 clearly necessary to test for plasticity: If significant plasticity had been present, the representation location
10 within a body part, in particular the lips, which was consistently stimulated with the same method, should
11 have changed from pre- to post-training; that was not the case. Also, the cortical distance between the
12 phantom digit and the stimulated stump location was not altered from pre to post training, although both
13 locations were stimulated with the same method.

14 The reported approach to effectively decrease PLP intensity, and daily fluctuations is simple to use and
15 can be applied in people with PLP with different clinical characteristics. It has been developed to specifically
16 provide substituting multimodal sensory stimulation (tactile and visual) to feedback volitional motor control
17 of the phantom limb. As the sensory stimuli are correlated with the residual limb muscular activity, which is
18 meant to control phantom movements, they provide a rather physiological way for improving the modulation
19 and motor control of the residual limb muscles during phantom movement execution. The technology used
20 can be set-up quickly and configured in a completely portable way. All the devices were wireless except for a
21 cable running from the vibrating band to its battery-powered controller. Therefore, using a small form factor
22 PC and video glasses, the entire experimental set-up can be fully portable. The patient software interface was
23 simple and intuitive, and no participant reported difficulty in understanding and executing the prescribed
24 exercises. This approach is also individualized as the operator interface contains several adjustable parameters
25 which can be easily tuned to match the participant's ability, resulting in a challenging but not frustrating

1 training experience. The entire hardware and software set-up was approved by the local ethics committee to be
2 used in a clinical setting as well as at the patient's home with indirect telephone supervision of an operator.
3 Therefore, this new treatment approach to reduce PLP can be exploited in a clinical setting, as described in the
4 current study, or used at home for chronic pain management.

5 *Training the volitional motor output of the residual limb muscles*

6 The multimodal sensory-motor training was effective in reducing PLP intensity while improving the
7 motor capacity of the trained muscles. The approach tested in this study addressed the remnant muscles of the
8 residual limb activated during the execution of different phantom movements (indifferently from the ability to
9 feel or move the phantom) as the participants trained using a visual and tactile feedback of the muscular effort
10 to execute these phantom movements. The motor control ability over the muscles of the residual limb
11 improved across the 12 effective training days as the participants were able to significantly improve the
12 amount of repetitions of the trained phantom limb movements of 167.85% on average from the first to the last
13 4 days of training. On the other hand the trained phantom limb movements, across the daily sessions, were
14 continuously increasing in speed as their time duration reduced of a 25.67 % from the first to the last 4
15 training days. Interestingly, considering the amount of accomplished movements and their duration in time, a
16 quick calculation of the overall trained time per session can be extracted to show the participants were able to
17 train ~10 min more (from 38.67 to 48.26 min per hour) during the final training period compared to the first 4
18 days of training as a consequent possible reduced fatigability in moving their phantom. Significantly
19 improved muscular effort and motor output control over the residual limb muscles controlling a phantom
20 movement can be drawn from the data on the tracking error which reduced of 14.73% on average from the
21 first to the last 4 training days.

22 *Effects on participants showing a blocked or telescoped phantom and with no referred fingers sensation*

23 A further analysis was conducted to study the effect of the training approach for the single participant
24 who showed no finger sensation during the initial assessment (subject 4, Table S1). The average PLP
25 intensity, acquired for the 4 days before starting the training (T1), and used as a reference value for PLP

1 intensity was compared to the average of the 4 days before ending the training (T2). A significant reduction (p
2 < 0.001) was reached as the initial PLP intensity of 4.9 ± 1.2 (mean \pm SD) decreased to 3.3 ± 0.4 .
3 Interestingly, the multimodal sensory-motor training significantly reduced the PLP intensity in this amputee
4 who referred no finger sensations even if part of his training was based on executing finger movements and an
5 intact 3D hand model was followed as reference regarding the type and timing of the movement to train
6 (making unlikely the embodiment of the 3D hand model (81, 82)). Possibly, in this case, the success in
7 reducing PLP depends on training the modulation of phantom limb motor execution and effort benefitting
8 from visual and tactile feedback originating from the motor activity meant to control the phantom limb (22).
9 More surprisingly, similar results were obtained for subjects 5, 6 and 9 (Table S1) who showed no movement
10 abilities for the phantom limb ($p < 0.019$, from 3.1 ± 0.8 to 2.3 ± 0.4 in subject 5; from 5.6 ± 0.5 to 4.9 ± 0.4 in
11 subject 6; from 5.6 ± 2 and 3.9 ± 1.5 in subject 9). Multimodal sensory-motor training was effective in
12 participants with an inability to move their phantom but who were still able to modulate the motor output of
13 the residual limb muscles. The approach tested in this study used the actual residual limb muscular effort as
14 feedback signal and the MVC EMG as reference polar plot (Fig. 1 B). Even in the absence of an activity that
15 involved the residual limb muscles, as it possibly happens for amputees with a frozen phantom (26), the tested
16 training led to improved motor control over these muscles, as reported for the significant reduction in the
17 tracking error and a positively correlated reduction in PLP intensity.

18 *Study limitations*

19 Besides the significant positive results achieved by the approach proposed in this study, some
20 limitations and drawbacks should be taken into consideration. The small number of trained participants and
21 the lack of a control group are limitations, both due to the high effort per subject (2 training-hours per day)
22 and in particular the availability of amputees with PLP who choose to take part in a three-week out-of-home
23 study. On the other hand the entire treatment set-up is based on easily controllable, low-cost commercially
24 available devices (PC and low-cost EMG-wrist band). The 8 electromagnetic micro-vibrators used for the
25 tactile stimulation, which represent a more expensive piece of equipment, can be easily replaced with cheap

1 micro electrical motors rotating an eccentric mass on the shaft (the same devices which provide vibrating
2 function in mobile phones). Therefore, in the future patients could train at home at the frequency that they
3 wish.

4

5 *Conclusion*

6 Using a multimodal sensory-motor training on phantom-limb movements with visual and tactile
7 feedback, we report a significant reduction in PLP intensity, which lasted for at least 30 days after the end of
8 the treatment. Although preliminary, this is the first study applying multimodal sensory feedback approach
9 (visual and tactile) to train the motor output of phantom movements leading to an average PLP intensity
10 reduction of 32.1%.

11

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20 Conception and design, Revising the manuscript; JH, Development of training tool, Acquisition of data,
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22 design, Revising the manuscript; MS, Acquisition of data, Analysis of data; PD, Design, Revising the
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Supplementary Material

QST Methods

QST (Rolke et al., 2006) was performed at T1 and T2 (see Table 3) including pressure and thermal pain thresholds, mechanical pain sensitivity, and two-point discrimination over four locations: (i) the above chosen stump trigger point, (ii) the corresponding finger of the intact side (reference for (i)), (iii) a medial spot on the

1 lateral aspect of the shoulder (3 cm below the acromion process) on the amputated side, (iv) the equivalent
2 location on the intact side (reference for iii.). The locations (i) and (ii) were marked and also used as
3 stimulation points for the fMRI evaluation. Demonstration of the procedures and familiarisation with the
4 stimuli over a location far from the testing sites (anterior aspect of the thigh contralateral the amputation side)
5 were executed before each test.

6 The thermal tests were executed before any mechanical test and always in the same order (Rolke et al., 2006).
7 They were executed with a Thermal Sensory Analyser II (Medoc, Israel). A 3 x 3 cm thermode at a baseline
8 temperature of 32°C, able to generate warm and cold stimuli with ramps of 1°C/s, (temperature range 0°C –
9 50°C), was placed over the tested region and fixed with a flexible band, whereas a stop button was given to
10 the participants free limb. Cold and warm detection thresholds (CDT and WDT) were assessed and the
11 thermal sensory limen (TSL) procedure (the difference limen for alternating cold and warm stimuli) was
12 executed before the thermal pain thresholds (Rolke et al., 2006). The ramped stimuli were terminated when
13 the participant pressed the stop button, as soon as cold or warm sensation was felt for the CDT and the WDT,
14 respectively. The cold pain and heat pain thresholds (CPT and HPT) were obtained asking the participant to
15 press the stop button as soon as the cold/warm stimulus changed into a painful one (with sharp, burning, or
16 stubbing characteristics). Thermal threshold means were calculated from three consecutive measurements.

17 The mechanical detection threshold (MDT) was measured using standardised Von Frey filaments exerting
18 forces upon bending, 2 s contact time, between 0.25 and 512 mN (graded with a 2 factor). An
19 ascending/descending paradigm was used where five threshold determinations were made, each with a series
20 of ascending and descending stimulus intensities. The final threshold was the geometric mean of these five
21 series (Rolke et al., 2006). Pin-Prick sensitivity (mechanical pain threshold, MPT) was assessed with specific
22 filaments with a flattened tip and a specified force (8, 16, 32, 64, 128, 256 and 512 mN) on the application.
23 Stimulus-response curves were assessed by a series of randomised stimuli, with each of the different probes (8
24 – 512 mN) repeated three times (Rolke et al., 2006). The participants had to indicate whether they perceived
25 the stimulus as dull or pricking/sharp, the latter indicative of the activation of nociceptive receptors, and to

1 assess stimulus intensity on a numerical rating scale from 0 - 100. Sensitivity to vibration was measured with
2 a standardised graded tuning fork (64 Hz, 8/8 scale). The vibration detection threshold (VDT) was evaluated
3 reporting the felt disappearance of the vibratory stimulus. The mean of three repetitions of threshold
4 disappearance was used. Pressure pain thresholds (PPT) were measured using a Pressure Algometer with a 1
5 cm² probe tip (Somedic, Sweden). Participants indicated when they perceived pain for the first time during
6 pressure stimulation with slowly increasing intensity (50 kPa/s). The stimulation stopped with the first report
7 of pain, the mean of three repetitions was registered as the PPT value. The maximum pressure intensity
8 applied was 1000 kPa.

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Subject	#	1	2	3	4	5	6	7	8	9	10
	Gender	M	F	F	M	F	M	F	M	F	M
Amputation	Age (years)	59	56	59	28	60	64	64	75	63	49
	Level	TRL	TRR	TRL	THL	TRL	TRR	TRL	THL	THL	THL
	Cause	Trauma	Trauma	Trauma	Trauma	Trauma	Trauma	Trauma	Vascular Disease	Trauma	Tumor
	Time since (years)	6.2	14.7	5.7	13.8	6.2	7.2	8.2	52.9	26.2	36.7
	Pain Before	N	N	Y	Y	Y	N	N	N	N	N
	PLP starting date	1 week	Immediate	2 months	1 year	2-3 months	1 week	2 weeks	few days	7 weeks	few days
	Stump length (cm)	26.5	10	17	26	18	16	15.5	19	19	27
PLP attacks	Frequency	Several times per day	Several times per day	No pain Attacks	Several times per day	No pain Attacks	No pain Attacks	Several times per week	Several times per day	Several times per day	Several times per day
	Intensity (VAS)	8	9.5	0	8.5	0	0	8	7	10	9.5
Pain	Duration (hours/day)	12	24	24	2.5	24	24	24	24	24	24
	Type	Stabbing, cutting	Burning, cramping, electric shocks, cutting	Cramping, electric shocks	Burning, electric shocks, cutting	Cramping, electric shocks, cutting	Cramping	Burning, cramping, freezing	Cramping electric shocks, stabbing, crushing	Burning, stabbing, cramping, electric shocks	Tingling, stabbing
	Medication	Lyrica 300 mg	Diclofenac Flector 5%, Novaminsulfon 500 mg	Lyrica 300 mg	Palexia 100 mg, Lyrica 125 mg	Lyrica 300 mg	Neurontin 600 mg, 1/3 Saroten Retard 75mg,	Tramal long 50 mg, Novaminsulfon 500 mg	No under pain medication	No under pain medication	No under pain medication
	Therapy	TENS, Acupuncture, Warming the stump	TENS, Warming the stump, Mirror therapy	TENS, Acupuncture	TENS, Warming the stump, Mirror therapy	TENS, Acupuncture, Yoga	TENS, Acupuncture, Mirror therapy	Massage	Massage, cold therapy	None	Ganglion removal
	Stump Pain (VAS)	0	2	0	3	0	0	0	0	0	0
	Description	All the fingers felt, slow movements control	All the fingers felt, slow movements control	All the fingers felt, hand compressed sensation	Distal portion of the arm and hand without finger distinction, slow movements control, cold sensation	All the fingers felt, not able to move, pressing sensation around the hand	All the fingers felt, not able to move, pressing sensation around the hand	All the fingers felt, slow movements control	All the fingers felt, slow movements control	All the fingers felt, not able to move, pressing sensation around the hand	All the fingers felt, slow movements control
Phantom	Telescoped (%)	0	0	0	0	0	100	75	0	20	10
	Unintentional Movements	N	Y	N	N	N	N	N	Y	Y	N
Prostheses	Type and daily use	Myoelectric, several hours	Myoelectric, several hours	Myoelectric, several hours	None	Myoelectric, several hours	Myoelectric, several hours	Myoelectric, several hours	Cosmetic, several hours	None	Myoelectric, entire day
	Intact hand use (% bimanual daily activities)	70	100	30	90	30	50	80	100	100	100

Table S1. Details of Participants. Telescoping has been calculated as the distance from the phantom fingertips to the stump (cm) / the length of the intact arm (cm) calculated from fingertips to olecranon in transradial and to humeral great tubercle in transhumeral. Stump, respectively. The stump length (cm) was measured starting from olecranon in transradial, from humeral great tubercle in transhumeral.

Legend:

- TRL, TRR = Transradial left, Transradial right

- THR, THL = Transhumeral left, Transhumeral right